Spectral screening to monitor the encapsulation yield and controlled release of molecules or cells

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INTRODUCTION AND OBJECTIVES

The most popular bioencapsulation matrices with biomedical applications are natural polymers (alginates, carragenans, pectins, chitosan) which protect and assure controlled release of lipophilic molecules (drugs, oils, vitamins, pigments) or cells (probiotics, human or animal cells) (Poncelet 2006; Socaciu 2008; De Vos 2009). Major advantages of such matrices are their biocompatibility, lack of toxicity, FDA authorization and controlled release of encapsulated structures in gastric and/or intestinal environment (Sabitha 2010).

Our previous studies were focused on optimization bioencapsulation of phytochemicals (manily functional oils) and probiotic cells into complex matrices based on alginate-chitosan-pullulan, to obtain microspheres with controlled density and release, fresh or freeze dried. A key problem remains the accurate evaluation of the incorporation rates, efficiency and yield, which needs non-distructive screening methods, fast and reliable (Rodriguez-Saona 2001; Socaciu 2009). According to our recent data we report here the use of optical spectroscopy, namely UV-Vis spectrometry and ATR-FTIR spectrometry to fingerprint the specific pattern of microspheres composition, to monitor the encapsulation yield and the release of the molecules and cells in different conditions.

MATERIALS AND METHODS

Seabuckthorn oil (SBO), a very rich source of functional lipids, carotenoids and vitamins is very labile, due to its high concentration in antioxidants and need efficient encapsulation. Different formulas of mixtures containing 10-12% SBO in alginate (AG) 0.5 to 2%, with/without chitosan (CH) were obtained, by ionotropic gelation technique resulting microspheres of 0.5-1 mm diameter (Trif 2007). The fresh microspheres were analysed as such or frozen under vacuum. The content and stability of the encapsulated oil was characterized by carotenoid spectral screening.

Four different probiotic cell types: *Lactobacillus plantarum, L.casei, Bifidobacterium breve, B.infantis,individual* or mixed were encapsulated in different proportions of sodium alginate (1 to 2%) and chitosan (1 to 2%), being characterized as such or after freeze drying. Their release, viability and survivability in simulated gastric (SGJ) and intestinal juice (SIJ) in specific buffered media were tested (Vodnar 2010; Pop 2012).

In both experiments, the spectral screening was realized by UV-Vis spectrometry (200-650 nm) using a Jasco V540 device and by ZnSe Attenuated Transmission (ATR) Fourier Transform Infrared Spectrometry (from 900 to 4000 cm^{-1}), using a Schimatzu IR Prestige - 21 FTIR spectrometer.

Both spectral investigations allowed the fingerprinting of the initial emulsions before encapsulation, the pattern and content of the encapsulated structures, the dynamic of the released molecules or cells in different conditions, modifications of the encapsulated analytes (e.g. oxidations or cell viability). Based on the spectral monitoring, there were calculated the Encapsulation Yields (EY) = Weight of microspheres/ expected weight of analyte + matrix and Release yield (RY) = the quantity of released in solvent/ encapsulated analyte.

RESULTS AND DISCUSSION

Studies on SBO microspheres

Fig.1 represents main data obtained from the encapsulation of SBO in alginate 2%. The spectral fingerprints UV-Vis (left) include specific peaks for lipids (210 nm), phenolics (280 nm) and carotenoids (450 nm). FTIR spectra of alginate 2% beads containing SBO (A), comparatively with alginate powder (B), non-encapsulated oil (C) and alginate 2% empty beads (D) (right)

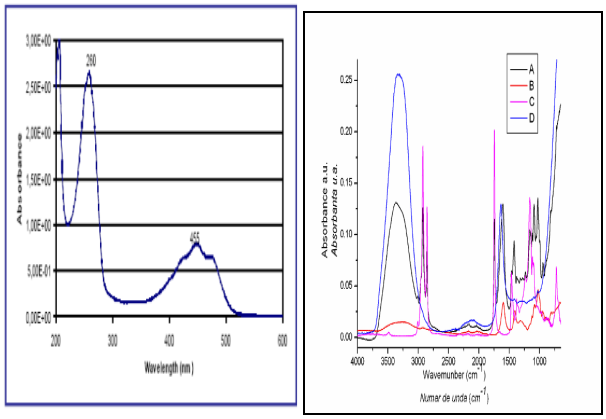

Figure 1. Spectral UV-Vis /FTIR fingerprints of microspheres containing SBO in alginate 2%.

Based on spectral data, the EY and RY values were calculated (Table 1).

Table 1 : Encapsulation (EY) and release yields (RY) of SBO encapsulated in AG or AG + CH matrices. The pH values for best release were selected.

SBO 10%	EY (%)	RY (%)	Best release
AG 0.5%	92.5	76.9	pH= 5.6
AG 1.5%	95.3	72.8	pH= 5.8-6
AG 1% +CH1%	96.8	65.4	pH= 5.8-7

Studies on encapsulated probiotics

Fig. 2. shows comparative FTIR spectral fingerprints (left) (500- 4000 cm^{-1}) of probiotics in alginate 2%: *L. plantarum, L.casei, B. breve, B.infantis,* 4-mix *vs* a Romanian mix. Right : Probiotics in chitosan (CH B) and alginate (AG B) microspheres *vs*. control empty beads (CH C, AG C) and *vs*. free probiotic mix (BF). The incorporation of bacteria inside the beads is visible at 2852 and 2956 cm^{-1}, and attributed to membrane lipids.

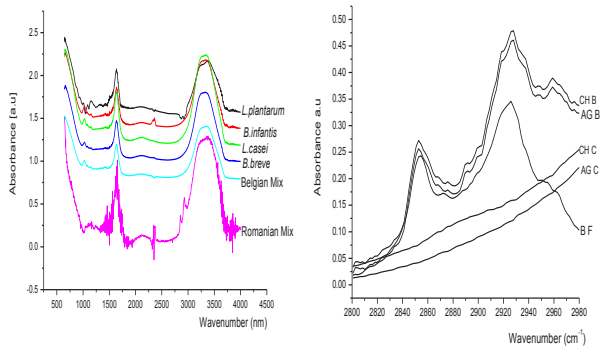

Figure 2. Comparative FTIR fingerprint of probiotics before (left) and after encapsulation (right). The identification of bacteria cells inside CH B and AG B microspheres was realized, *vs* Controls (CH C, AG C) and *vs* FB.

Based on the UV-Vis and FTIR spectral screening, the effect of AG and CH concentrations on the release and survival of probiotics, were determined, as shown in Fig.3.

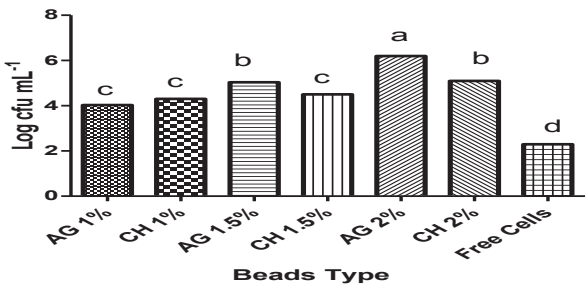

Figure 3. Effects of matrix type (AG or CH) and concentration on survival of encapsulated probiotics after succesive incubation in SGJ and SIJ. Values with different letters (a,b,c,d) have statistical significance (P<0.05).

CONCLUSIONS

By different experimental studies there were found optimized formulas to encapsulate seabuckthorn oil and probiotics, into natural composites, mainly alginate and chitosan. To monitor adequately the efficiency and yields of encapsulation and release, the UV-Vis and FTIR spectrometry proved to be adequate and reliable methods, essential for a correct and fast monitoring of stability and release.

The experimental data demonstrate the utility of monitoring by spectral screening which need to be correlated with SEM microscopy, Raman microspectrometry or spectroscopic imaging. All these complementary analytical tools are needed for a reliable evaluation of controlled delivery to targeted organs *in vivo*, as modern technologies for molecular medicine-based therapy.

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